IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BOARD OF PATENT APPEALS AND INTERFERENCES

In re:

Applicant: Kent, et al.	)	
Serial No.: 09/710,633	)	Group Art Unit: 1654
Filed: November 8, 2000	)	Examiner: Russel, J.
Title: SYNTHESIS OF PROTEINS BY NATIVE CHEMICAL LIGATION	)	Our Ref.: TSRI 478.0Con1

Supplemental Appeal Brief

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

The Applicant appeals the Final Office Action, dated 12/08/2003, and the rejection of claims 11-14 and 32 therein. This Supplemental Appeal Brief is being submitted responsive to the Notification of Non-Compliant Appeal Brief, dated 01/03/2006.

Real Party in Interest

The present application has been assigned by all inventors to the Scripps Research Institute, which is the real party in interest.

Related Appeals and Interferences

There are no related appeals or interferences.

Status of Claims

Claims 11-14 and 32 are rejected.

Claims 8, 10, 24, 26, and 29-31 are allowed.

Claims 1-7, 9, 15-23, 25, 27-28 are canceled.

Claims 11-14 and 32 are on appeal.

Status of Amendments

No amendments after final rejection have been filed. All prior amendments have been entered.

Summary of Claimed Subject Matter

Proteins and/or protein domains having native peptide backbones are produced by a method of native chemical ligation. Native chemical ligation employs a chemoselective reaction between a first oligopeptide and a second oligopeptide. The first oligopeptide includes a C-terminal thioester; the second oligopeptide includes an N-terminal cysteine amino acid residue capable of forming a β-aminothioester linkage with the C-terminal thioester on the first oligopeptide. Upon admixing, the first and second oligopeptides produce the transient β-aminothioester-linked intermediate. The transient β-aminothioester-linked intermediate then spontaneously undergoes a rearrangement to provide the full length ligation product having a native peptide bond at the ligation site. The technique of native chemical ligation is employable for chemically synthesizing full length proteins and protein domains.

An important aspect of the invention is that the claimed method may be employed for synthesizing a derivative of a naturally isolatable protein containing one or more variant or cysteine residues that are not found in the naturally isolatable

protein. Support in the Specification for a derivative of a naturally isolatable protein containing one or more variant or cysteine residues is found in Figure 9 and in the Brief Description of Figures, page 21 of the Specification after line 26, wherein Figure 9 is described as a reversed-phase HPLC tracing for **HIV-1 K41**. Support in the Specification for the use of the claimed synthetic method for making a derivative of a naturally isolatable protein containing one or more variant or cysteine is found generally in Example 4, pages 37, third paragraph - page 38, first paragraph, wherein the synthesis of **HIV-1 K41** is described. Further support for this aspect of the invention is found in the Specification at page 7, lines 16-19, and at page 21, lines 12-12-14.

An other important aspect of the invention is that the claimed method may be employed for synthesizing a mammalian protein, human proteins, and/or cytokines. Support for this aspect of the invention is found in Examples 2 and 3, i.e., pages 31, third paragraph - page 37, second paragraph, wherein the synthesis of human cytokines IL3 and IL8 are described. Further support in found in the Specification at pages 16, second paragraph - page 18, first paragraph, in Figures 5, 6, 7, and 8, and in the Brief Description of Figures for Figures 5, 6, 7, and 8.

Grounds of Rejection to be Reviewed on Appeal

Claims 11-14 and 32 were given a final rejection under 35 U.S.C. 112, first paragraph, on the basis that they contain subject matter which is not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. This basis for rejection can be subdivided into five issues, viz.:

First Issue: Does the specification support a disclosure of a derivative of a naturally

isolatable protein containing one or more variant or cysteine residues that are not found in the naturally isolatable protein?

Second Issue: Does the specification support a disclosure that the claimed method of native chemical ligation may be employed to synthesize a derivative of a naturally islolatable protein containing one or more variant or cysteine residues that are not found in the naturally isolatable protein.

Third Issue: Does the specification support a disclosure that the cysteine residue substituted for a naturally-occurring residue in Figure 9 is a general reaction technique which can be extrapolated to the synthesis of proteins in general using native chemical ligation?

Fourth Issue: Did Applicant make an admission against interest in the Specification with the statement at page 6, lines 1-3. More particularly, did Applicant's statement that the invention "raises the exciting prospect" of unrestricted variation of protein covalent structure indicate that the inventor did not have in his possession the claimed invention at the time the application was filed.

Fifth Issue: Does the disclosure in Figure 9 that native chemical ligation may be employed to synthesize HIV-1 protease having a cysteine residue substitution support the use of native chemical ligation to synthesize mammalian proteins, human proteins, and cytokines having a similar cysteine residue substitution?

Arguments

First Issue:

Does the specification support a disclosure of a derivative of a naturally isolatable protein containing one or more variant or cysteine residues that are not found in the naturally isolatable protein?

Response:

A naturally isolatable protein containing a cysteine residue that is not found in the naturally isolatable protein is disclosed in the specification at Example 4, pages 37-38 and illustrated in Scheme 9 on page 39. The title atop Scheme 9 indicates that the scheme illustrates a "**Mutant HIV-1 K41 Protease Synthesized by Native Chemical Ligation.**" It would well understood by one skilled in the relevant art that the term "Mutant HIV-1 **K41** Protease" means that a mutation has occurred at position **41** and that, within the naturally isolatable form of HIV-1 Protease, position **41** is normally occupied by a lysine, i.e., **K41**. To say that the HIV-1 Protease is a "mutant" means that one or more amino acid residues of the naturally isolatable form of the protein have been replaced or substituted by another amino acid residue, i.e., the lysine at position **41** of naturally isolatable form of HIV-1 Protease has been replaced or substituted with another amino acid residue other than lysine. The particular substitution is shown on the right hand side of Scheme 9 wherein the oligopeptide 41-49 is shown to have an N-terminal cysteine amino acid residue. The 41-49 oligopeptide is then shown to undergo a native chemical ligation with the 1-40 oligopeptide so as to form the 1-99 product having a cysteine at position **41**. The 1-99 product was characterized by electrospray mass spectroscopy, as described in the specfication at the top of page 38 and illustrated in Scheme 9, and shown to have the expected mass shift as compared to naturally isolatable form of HIV-1 protease resulting from the substitution of the

lysine at position **41** with a cysteine residue at the same position (compare mass spectrum on the right side of Scheme 9 with the inset therein).

The Board of Appeal is requested to reverse the Examiner's finding that Applicant's specification does not support a disclosure of a derivative of a naturally isolatable protein containing one or more variant or cysteine residues that are not found in the naturally isolatable protein.

Second Issue:

Does the specification support a disclosure that the claimed method of native chemical ligation may be employed to synthesize a derivative of a naturally islolatable protein containing one or more variant or cysteine residues that are not found in the naturally isolatable protein?

Response:

The Examiner states the following:

> "The original disclosure does not include the concept of altering a naturally-occurring protein's amino acid sequence by replacing amino acids with variant residues or cysteine residues or by inserting cysteine residues into the amino acid sequence so that a derivative of the naturally-occuring protein can be synthesized by the disclosed method." (Final Office Action, dated 12/08/2003, page 2, bottom of first paragraph)

The Examiner's restatement of Claims 11 and 32 is open to ambiguity. Claims 11 and 32 are directed to a method for synthesizing a derivative of a naturally isolatable protein wherein the derivative contains one or more variant or cysteine residues that are not found in said naturally isolatable protein.

Support in the specification for Claims 11 and 32 is provided as follows:

> "The general synthetic access provided by the method of native chemical ligation greatly expands the scope of variation of the covalent structure of the protein molecule." (Specification, page 7, bottom of first paragraph.)
>
> "It [native chemical ligation] provides for unrestricted variation of protein covalent structure made possible by general synthetic access, and provides new impetus to exploration of the structural basis of properties such as folding, stability, catalytic activity, binding, and biological action." (Specification, page 21, second paragraph.)

Detailed support in the specification for Claims 11 and 32 is found in Example 4, viz., the mutant HIV-1 K41 Protease, as discusses above (Specification, pages 37-38 and Scheme 9 on page 39).

The Board of Appeal is requested to reverse the Examiner's finding that Applicant's specification does not support a disclosure that the claimed method of native chemical ligation may be employed to synthesize a derivative of a naturally islolatable protein containing one or more variant or cysteine residues that are not found in the naturally isolatable protein.

Third Issue:

Does the specification support a disclosure that the cysteine residue substituted for a naturally-occurring residue in Figure 9 is a general reaction technique which can be extrapolated to the synthesis of proteins in general using native chemical ligation?

Response:

Applicant's specification discloses that an *N*-terminal Cys residue is necessary for native chemical ligation:

> "As disclosed herein, native chemical ligation is limited to reaction at an *N*-terminal Cys residue." (Specification, page 14, lines 21-23)

The necessity of the Cys residue is further established in Scheme 5 where the control reaction having no *N*-terminal Cys residue failed to proceed.

The Examiner states that "there is no description which indicates that this cysteine residue [i.e., the *N*-terminal Cys] has any particular importance to the inventors, . . ."

Applicant traverses this statement. Support in the specification for the importance of the *N*-terminal Cys is provided as indicated above and repeated throughout the specification. The *N*-terminal Cys is central to the invention.

Applicant's specification also discloses that the native chemical ligation reaction is of general applicability. More particularly, the specification discloses that the native chemical ligation reaction may be employed with oligopeptides having **any of the full range of functional groups normally found in proteins**, just so long as one oligopeptide includes a *N*-terminal residue and the other oligopeptide includes a C-terminal thioester:

> "Several model peptides have been synthesized by the method of native chemical ligation. The successful synthesis of these model peptides establish that native chemical ligation is generally applicable to peptides containing the full range of functional groups normally found in proteins. Even free internal Cys residues may be present in either of the reaction segments. . . ." (Specification, page 14, lines 10-16.)

As indicated above, the specification teaches that the native chemical ligation is "generally applicable to peptides containing the full range of functional groups normally found in proteins."

The Board of Appeal is requested to reverse the Examiner's finding that the specification does not support a disclosure that the cysteine residue substituted for a naturally-occurring residue in Figure 9 is a general reaction technique which can be extrapolated to the synthesis of proteins in general using native chemical ligation.

Fourth Issue:

Did Applicant make an admission against interest in the Specification with the statement at page 6, lines 1-3. More particularly, did Applicant's statement that the invention "raises the exciting prospect" of unrestricted variation of protein covalent structure indicate that the inventor did not have in his possession the claimed invention at the time the application was filed.

Response:

The source of the Examiner's allegation that Applicant made an admission against interest is found in the Background section of the Specification, viz.:

> "Straightforward total chemical synthesis of proteins represents the realization of an important objective of organic chemistry. It raises the exciting prospect of unrestricted variation of protein covalent structure made possible by general synthetic access, and will give new impetus to exploration of the structural basis of properties such as folding, stability, catalytic activity, binding and biological action." (Specification, Background Section, bottom of page 5 and top of page 6.)

The Background reference to the "exciting prospect of unrestricted variation of protein covalent structure" is not an admission that the inventor did not have in his possession the claimed invention at the time the application was filed. The context of the remark indicates that the term "exciting prospect" was employed by Applicant and would be understood by persons skilled in the art as mere conjecture concerning the likely reaction by persons skilled in the art to the disclosure of Applicant's native chemical ligation method. Applicant was merely speculating that his invention would be

received with "excitement" by persons skilled in the art because persons skilled in the art would understand that they could employ the invention to explore unrestricted variation of protein covalent structure.

The Board of Appeal is requested to reverse the Examiner's finding that Applicant make an admission against interest in the Specification with the statement at page 6, lines 1-3.

Fifth Issue:

Does the disclosure in Figure 9 that native chemical ligation may be employed to synthesize HIV-1 protease having a cysteine residue substitution, when taken in context with the remainder of the specification, support the use of native chemical ligation to synthesize mammalian proteins, human proteins, and cytokines having a similar cysteine residue substitution?

Response:

The HIV-1 protease disclosed in Figure 9 is a human viral protein. Although it is encoded by a virus, it is synthesized *in vivo* using human protein biosynthetic pathways.

More importantly, however, Applicant's specification discloses that the claimed method for native chemical ligation is equally effective with model peptides, human proteins, bacterial proteins, and viral proteins. Indeed, as indicated above with respect to model peptides, the specification teaches that "native chemical ligation is generally applicable to peptides containing the full range of functional groups normally found in proteins." Accordingly, the claimed method for native chemical ligation can be equally employed with any protein or protein domain "containing . . . functional groups normally found in proteins."

The Board of Appeal is requested to reverse the Examiner's finding that Applicant's disclosure in Figure 9 that native chemical ligation may be employed to synthesize HIV-1 protease having a cysteine residue substitution, taken in context with the remainder of the specification, does not support the use of native chemical ligation to synthesize mammalian proteins, human proteins, and cytokines having a similar cysteine residue substitution.

Summary:

Applicant respectfully requests that the Board reverse the Examiner's final rejection of claims 11-14 and 32 under 35 U.S.C. 112, first paragraph, and the Examiner's finding that claims 11-14 and 32 contain subject matter which is not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. Applicant further requests that the application be returned to the Examiner for further examination.

Respectfully submitted,

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CLAIMS ON APPEAL
(Claims 11-14 and 32)

Listing of Claims on Appeal:

Claim 11: The method of claim 8, wherein said desired protein is a derivative of a naturally isolatable protein that contains one or more variant residues that are not found in said naturally isolatable protein.

Claim 12: The method of any of claims 10 or 11, wherein said protein is a mammalian protein.

Claim 13: The method of claim 12, wherein said mammalian protein is a human protein.

Claim 14: The method of claim 13, wherein said human protein is a cytokine.

Claim 32: A method for producing a desired protein or domain thereof, which comprises admixing:

(I) a first oligopeptide, said first oligopeptide comprising a fragment of said desired protein or domain thereof, and having a C-terminal thioester; and

(II) a second oligopeptide, said second oligopeptide comprising a fragment of

said desired protein or domain thereof, and having an N-terminal cysteine amino acid residue having an unoxidized sulfhydryl side chain and a free amino group that is capable of forming a β-aminothioester linkage with said C-terminal thioester that rearranges to form an amide bond therein between;

wherein said admixing is conducted under conditions sufficient to permit the formation of an amide bond between the C-terminus of said first oligopeptide and the N-terminus of said second oligopeptide;

wherein said desired protein is a derivative of a naturally isolatable protein, said desired protein containing one or more cysteine residues that are not found in said naturally isolatable protein.

EVIDENCE APPENDIX

none

RELATED PROCEEDINGS APPENDIX

none